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A NONBITUNICATE ASCUS IN THE ASCOSTROMATIC GENUS *ASTERINA*

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ABSTRACT — The ascus of *Asterina carbonacea* Cooke is nonbitunicate and is formed in an ascostroma. There is a similarity with the eu-archaeascé ascus type. A comparison of this ascus type with the bitunicate ascus demonstrates a difference in the wall structure and the origin of the extended wall formed during spore ejaculation. The primary ascus wall stains blue with IKI and Sudan Black B ; pretreatment with KOH before IKI results in a nonstaining primary wall and blue-staining of the secondary wall. The banded pattern of the secondary wall is recognizable as Couche D1 and Couche D2. Couche D2 extends in ascospore dispersal from an apically positioned dome-shaped wall component into a tube reaching to the surface of the ascocarp. None of the ascus wall layers separate during spore dispersal. This study indicates that the family Asterinaceae should be recognized as a taxon apart from those included in the loculoascomycetes.

RÉSUMÉ — L'asque d'*Asterina carbonacea* Cooke est non-bituniqué et est formé dans un ascostroma. Il présente une similitude avec le type eu-archaeascé. La comparaison avec l'asque bituniqué montre une différence dans la structure de la paroi et dans l'origine de celle qui se forme, par extension, pour l'éjection des spores. La paroi primaire de l'asque se colore en bleu avec l'IKI et le noir Soudan B ; un prétraitement par KOH avant l'IKI ne donne pas de coloration de la paroi primaire, mais une coloration bleue de la paroi secondaire. On peut reconnaître les couches D1 et D2 dans la paroi secondaire. Lors de la dispersion des ascopores, la couche D2 s'allonge, à partir d'une zone apicale en forme de dôme, en un tube qui rejoint la surface de l'ascocarpe. Aucune des couches de la paroi ascale ne se sépare pendant les processus d'éjection des spores. Cette étude indique que la famille des Asterinaceae doit être reconnue comme un taxon particulier parmi les loculo-ascomycètes.

KEY WORDS : *Asterina*, ascostromatic ascomycete, ascus.

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INTRODUCTION

The ascus has a wide variance in structure (SHERWOOD, 1981 ; ERIKSSON, 1981). HAWKSWORTH & al. (1983) noted that the recognition of only the bitunicate, prototunicate, and unitunicate major ascus types in the last decade is too simplistic an approach to ascus characterization.

The development of an *Asterina* species was shown by WARD (1882) to be ascostromatic in that the ascocarp developed before the origination of asci from an included hyphal system that formed a « boss or raised disc ». The asci were illustrated as uniformly thin-walled.

The ascus of *Asterina melastomatis* Léveillé was illustrated by ARNAUD (1918) as having a thickened apical region with the tube-shaped extension of the protoplast ; ascospores were shown reaching the outer surface of the ascocarp hymenium in linear form from an extended thin-walled cupulate enclosure that appears to be intended as the expended ascus. The asci of *Asterina mulleri* Stevenson (STEVENSON, 1943) and *A. solanacearum* Orejuela (OREJUELA, 1944) were described as « evanescent ».

The family of the species studied here, the Asterinaceae Hansford, has been described as having a « probably semifissitunicate » or « rostrum-like » jack-in-the-box ascus (ERIKSSON, 1981). ERIKSSON (1981) described the rostrate type of ascus as having « wall that is « reverted and extruded as a rostrum ». The semifissitunicate type of ascus was described as having a « reverted » inner wall which extended as a « rather long rostrum ».

The Asterinaceae ascus characterization was composed by ERIKSSON (1981) from a description of the ascus of the assumed type species of the family type genus ; « type species not indicated, but commonly cited as *A. melastomatis* Léveillé ». The material studied was not the type material from Brasil (LÉVEILLÉ, 1845), but rather Guatemalan material collected in 1907 and distributed as # 1749 in Rehm's ascomycete exsiccatum. The inner wall was said to be distinct and staining IKI+ blue and Congo Red+ as minute granules on the ascus surface. The inner wall was described as a layered, up to approximately 6 µm thick, « apical dome » which showed an « ocular chamber » and « Cobolt Blue+ meniscus.

The ascus of the asterinaceous *Placoasterella baileyi* (Berkeley & Broome) Arx was examined with electron microscopic resolution by TYSON & GRIFFITHS (1976b). They found the banded pattern secondary wall and compared the structure to that found by REYNOLDS (1971). The ascus was observed to undergo an elongation of the secondary wall prior to losing a basal attachment in the ascocarp. The entire ascus and the contained ascospores of this species were found to be discharged from the ascocarp with separation of the wall into two layers ; no actual ascospore discharge was seen by these authors, but a « mechanism involving the dissolution of the thin ascospore-enclosing sheath, and the passive release of ascospores » was suggested. ERIKSSON (1981) pronounced the dehiscence of the ascus of *P. baileyi* as « pseudofissitunicate » after the false Jack-in-the-Box ascus of PARGUEY-LEDUC & CHADEFAUD (1963) ; the ascospores are extruded in the uppermost part of the epiplast with the surrounding plasmalemma in this mode of dehiscence.

MATERIALS AND METHODS

The ascus used in this study is that of *Asterina carbonacea* Cooke.

The material used in this study is curated in Herbarium LAM as # 300451. The collection was made by Allen G. SHUEY from a cypress swamp located 1 mile west of US Highway 441/17-92 and 4.5 miles north of the Orange-Osceola county line, between Kissimmee and Orlando, Florida, Section 16, Township 24S, Range 29E, on 5 February 1980. The fungus was growing on the surface of the leaves of the swamp red bay, *Persea palustris* (Raf.) Sarg.

Light microscope observations were made with a Zeiss dissection microscope and a Nikon compound microscope. Whole and squash mounts and handcut cross sections were made of the ascocarps. The hymenial layer was dissected from KOH pretreated ascocarps. Final mounts for observations were made in lactophenol. Cytochemical tests were made according to the protocol of ERIKSSON (1981) and BARAL (1987).

Transmission electron microscope observations were made with a JOEL 100 CXII electron microscope at 80 KV. Fruit bodies were teased off the surface of a leaf into a 3% KOH solution and allowed to soak for approximately ten minutes. The material was then fixed in 2% glutaraldehyde and 2% paraformaldehyde mixed with a 0.1 M cacodylate buffer containing 0.02% CaCl2. The material was rinsed with the buffer after 24 hours in the fixative, and then rinsed with 30% ethanol. The ascocarps were soaked in 50% ETOH containing 0.5% uranyl acetate for 12 hours. Dehydration was carried out in a series of 80%, 95%, and 100% ETOH and in 100% propylene oxide. The infiltration of the embedding material began in a 12-hour soak in a 50/50 ratio of propylene oxide and Spurr's medium. Final embedding was carried out in the medium-hard formula of the Spurr's epoxy. A Sorvall MT2-B ultramicrotome equipped with a diamond knife was used to produce approximate 0.75 mm thick sections for review with the light microscope and approximate 800 angstrom sections for TEM viewing. Staining of the thin sections was done with an aqueous saturated solution of uranyl acetate, followed by the lead citrate stain. Scanning electron microscope observations were made with a Cambridge S4-10. Dried leaf specimens with fruit bodies were coated with gold-platinum in a sputterer coater.

RESULTS

The life cycle (Fig. 1) begins with the germination of the two-celled ascospore (Figs. 1 A-B) on the leaf surface. An initial hypha is formed in usually the apical area of one ascospore cell (Fig. 1 B) ; branching is apparent after the formation of only a few cells (Fig. 1 C). The hyphopodia begin to appear soon thereafter (Fig. 1 D).

The ascocarp is comprised of a pair of hyphal systems which make up what is essentially a fruitbody with indeterminate growth. The ascocarp originates, apparently at random, from a hyphal cell of the darkly pigmented foliicolous mycelium (Figs. 1 E, 2 A). Resultant growth progresses in a plane parallel to the substrate surface. Divisions of the first and a few subsequent cells initiate prescribed meristematic activity. The first cells are somewhat irregularly arranged as a flattened mass (Fig. 1 F). A peripheral meristematic zone is established by cell division in

cohesive hyphal tips (Figs. 1 G-I). The lead cell of each component hyphal strand gives rise to one to several cells which diverge regularly into the upper hyphal system and irregularly into a lower hyphal system.

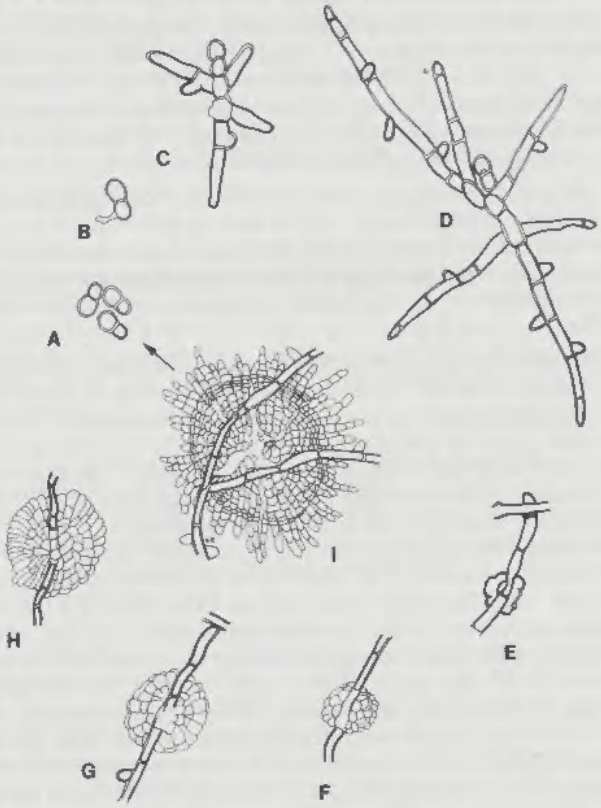
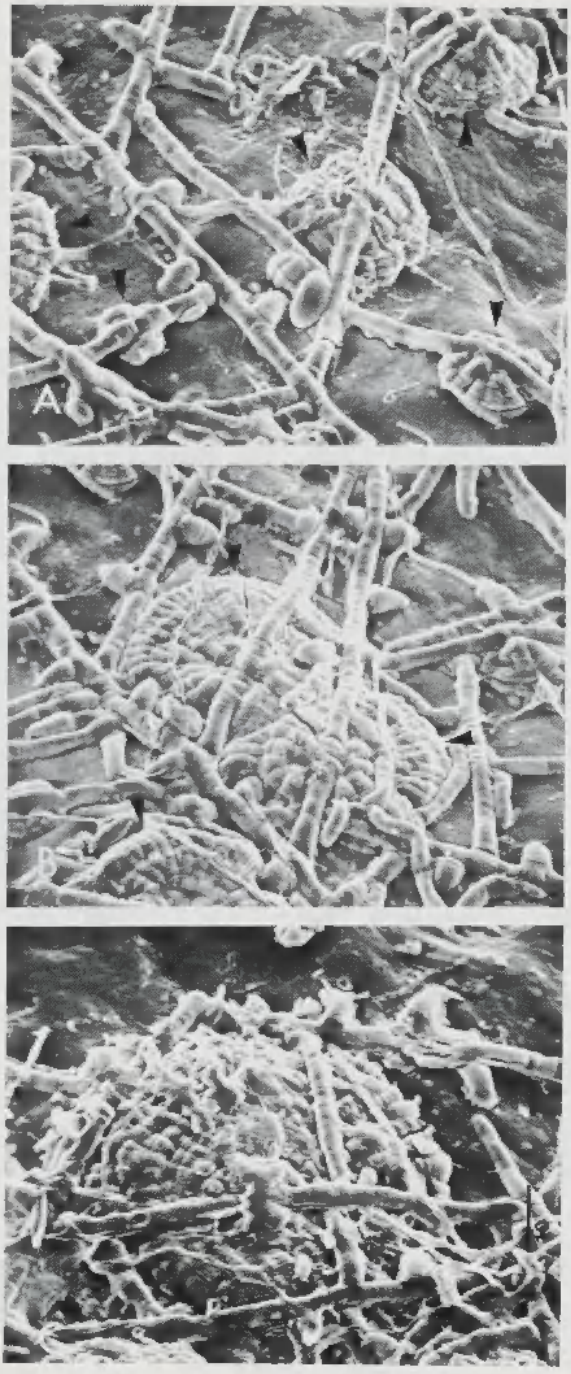


Fig. 1 — *Asterina carbonacea* Cooke. Life history. A. Three bicelled ascospores. B. Ascospore with germ tube. C. Initial hypha of young mycelium with three branches. D. A more advanced mycelium with hyphopodia on some component hypha. E. Hypohyphal growth preliminary to ascocarp formation. F. Young ascocarp initial. G-H. More advanced ascocarp stages with radiate pattern of upper hyphal system layer evident. I. Mature ascocarp with ruptured upper layer and continued growth of individual hyphal strands at the ascocarp edge.

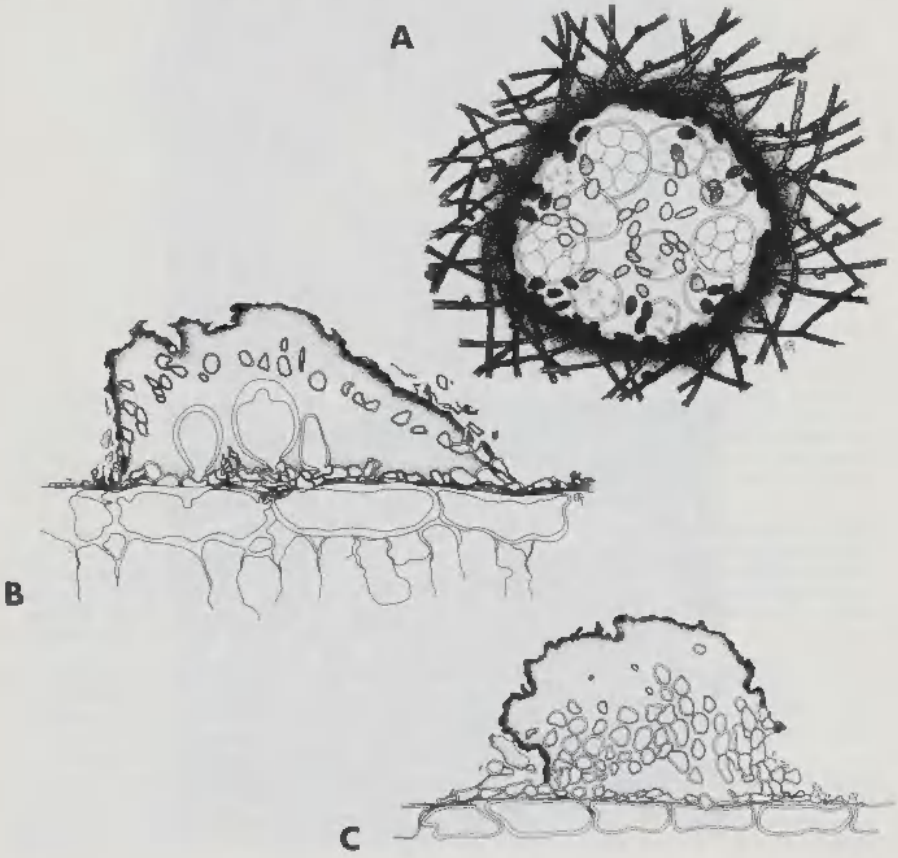
Fig. 1 — Cycle d'*Asterina carbonacea* Cooke. A. Trois ascospores bicellulaires. B. Ascospore avec tube germinatif. C. Hyphes initiales de mycélium jeune avec 3 ramifications. D. Mycélium plus âgé avec des hyphopodes sur quelques hyphes. E. Croissance hypohyphale précédant la formation de l'ascocarpe. F. Début d'un ascocarpe. G-H. Stades d'ascocarpes plus avancés montrant l'orientation rayonnante de la couche hyphale supérieure. I. Ascocarpe mûr montrant une rupture de la couche supérieure et la croissance continue des hyphes individuelles sur les bords.

Fig. 2 — *Asterina carbonacea* Cooke. Views of the ascocarp (S.E.M.). A. Four ascocarps (arrows) in the initial stages of formation from beneath the hyphal strand of origin. B. Ascocarps (arrows) at a more advanced stage with the radial pattern of upper hyphal system layer evident. C. Mature ascocarp with intact upper hyphal system layer. Note the hyphopodiate hyphae traversing the leaf surface and the ascocarp surface as well.

Fig. 2 — Ascocarpe d'*Asterina carbonacea* Cooke (M.E.B.). A. Premiers stades de formation de 4 ascocarpes (flèches) sous les hyphes initiales. B. Ascocarpes (flèches) à un stade plus avancé montrant l'orientation rayonnante de la couche hyphale supérieure. C. Ascocarpe mûr montrant une couche hyphale supérieure intacte. Noter l'hyphe hyphopodiale traversant la surface de la feuille et celle de l'ascocarpe.



An unmagnified dorsal view of the upper hyphal system shows a small, rounded, black growth on the surface of a leaf. Low magnification demonstrates the radial pattern of the component darkly pigmented hyphae which branch at more-or-less regular intervals so that a tissue-like confluence is maintained with increasing diameter of the fruitbody (Figs. 1 H-I, 2 B-C). The synchronized division results in a concentric pattern of surface ridges (Fig. 2). The upper layer maintains its integrity until the pressure from the contained maturing asci, beginning their discharge, causes it to break into deciduous segments in larger-sized fruit bodies (Fig. 1 I). Separations between hyphal strands occur at several points in the central older portion of the ascoma and radiate outward toward the ascocarp edge. The resultant stellate pattern of the split, pigmented, upper hyphal system is visually enhanced because of the exposed underlying non-pigmented lower hyphal system. The angular flaps of pigmented hyphae initially remain intact as additional peripheral meristematic activity and a corresponding maturing of the central area hymenium occur. Further disintegration of the stellately fractured upper layer into small bits of tissue is usually associated with the older ascocarp. Both mature asci and developing asci become apically exposed with only a few hyphal strands running over the surface of the ascocarp (Fig. 3 A-B). At this stage vertically oriented portions of the upper layer remain near the outer ascocarp edge (Fig. 3 C).



The lower hyphal system is the source of the asci and of secreted gelatineous material. This system is first apparent when the upper hyphal system reaches a diameter in the range of 300 μm (Fig. 4). Copious production of a hygroscopic mucin coincides with the formation of primary branches directly from the peripheral meristem zone at irregular intervals. Toluidine blue staining of thin sections and of squash mounts results in a metachromatic reaction of the gel ; it becomes an intense purple to light lavender. The non-pigmented hyphae of the lower hyphal system ultimately form a loose mycelial network under the overlying upper hyphal system. Secondary branches develop from outgrowths of the primary hyphal cell at close intervals.

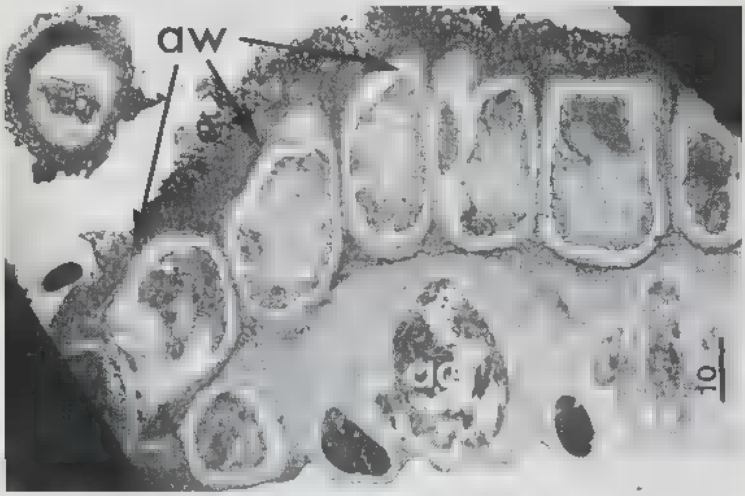


Fig. 4 — *Asterina carbonacea* Cooke. Longitudinal section through the ascocarp (T.E.M.). The upper hyphal system forms the shield shaped ascocarp wall (aw). The ascus initials (ac) originate from the lower hyphal system. A layer of gelatinous material pervades the entire ascocarp.

Fig. 4 — Coupe longitudinale de l'ascocarpe d'*Asterina carbonacea* Cooke (M.E.T.). Le système hyphal supérieur forme la paroi de l'ascocarpe en forme de bouclier (aw). Les initiales des asques (ac) se forment à partir du système hyphal inférieur. Une couche de matériel gélatineux occupe l'ascocarpe tout entier.

Fig. 3 — Schémas de l'ascocarpe d'*Asterina carbonacea* Cooke. Vue apicale montrant que le réseau hyphal supérieur du bouclier est désagrégé à l'exception de quelques cellules restant à la surface d'une matrice gélatineuse. On voit des asques immatures et des asques contenant les ascospores. B. Coupe longitudinale au milieu d'un ascocarpe mûr à la surface d'une feuille. Le réseau hyphal supérieur formant le bouclier et le réseau hyphal inférieur ascogène sont immergés dans une matrice gélatineuse. C. Coupe longitudinale sur les bords d'un ascocarpe mûr. Le système hyphal supérieur est orienté verticalement sur les bords de la masse hyméniale.

Fig. 3 — *Asterina carbonacea* Cooke. Diagramatic views of the ascocarp. A. Aerial view of the upper hyphal system forming the shield has disintegrated except for a few cells remaining on the surface of the inapparent gelatineous matrix. Both immature asci and asci with ascospores are illustrated. B. Longitudinal midsection through mature ascocarp on leaf surface. The upper hyphal system forming the shield and the lower hyphal system forming the ascogenous system are immersed in a gelatineous matrix. C. Longitudinal section through mature ascocarp at its edge. The upper hyphal system is vertically oriented at the edge of the contained hymenial area.

Fig. 5. — *Asterina carbonacea* Cooke. Ascus in light microscope view. A. Young ascus cell in initial growth stage. Arrows indicate primary wall. B. Preasco-sporogenesis ascus with mature wall [na = apical chamber, site of the nasse apicale]. C. Mature ascus with bicelled ascospores [•].

Fig. 5 — Asque d'*Asterina carbonacea* Cooke en microscopie optique. A. Jeune cellule initiale d'asque. Les flèches indiquent la paroi primaire. B. Phase préascosporogène d'un asque à paroi mûre [na = chambre apicale, site de la nasse apicale]. C. Asque mûr avec des ascospores bicellulaires [•].

The sympodially produced asci originate at irregular intervals from the secondary branches. The ascus initial cell enlarges somewhat in width, but mostly in length. Growth in the initial stages is accompanied by the deposition of wall material in an encompassing primary layer (Fig. 5 A). A single nucleus can be demonstrated in the cell at this stage with phase microscopy in both recently collected and KOH reconstituted dried material.

The formation of the secondary ascus wall is apparent when the ascus initial cell reaches a height of 60-70 µm (Fig. 5 B). This material forms an apical dome in the apex of the ascus which has a conspicuous extension of the protoplast into its center (Figs. 5 B-C). The longitudinally oriented striae of the nasse apicale can be demonstrated with the light microscope as was done previously (REYNOLDS, 1971). The striae are somewhat difficult to see with the ascus in midfocus in lactophenol but are discernable when the ascus is stained with IKI, both with and without KOH pretreatment. They also become better seen with a slight distorsion of the ascus apex in a microscope mount ; the striae are more prevalent in the ascus with formed ascospores and before pigmentation develops in the ascospore wall.

A single nucleus can be demonstrated in the cell at this stage with phase microscopy. The ascus undergoes a change in its shape during ascosporogenesis resulting in a different morphology of the secondary wall material in the ascus apex and in the overall outline of the ascus (Figs. 5 B-C).

The primary ascus wall, illuminated as a heavy line in Figs. 9 C-D, stains blue in Sudan Black B. The results with dilute and strong solutions of IKI and Meltzer's solution are given in Table 1.

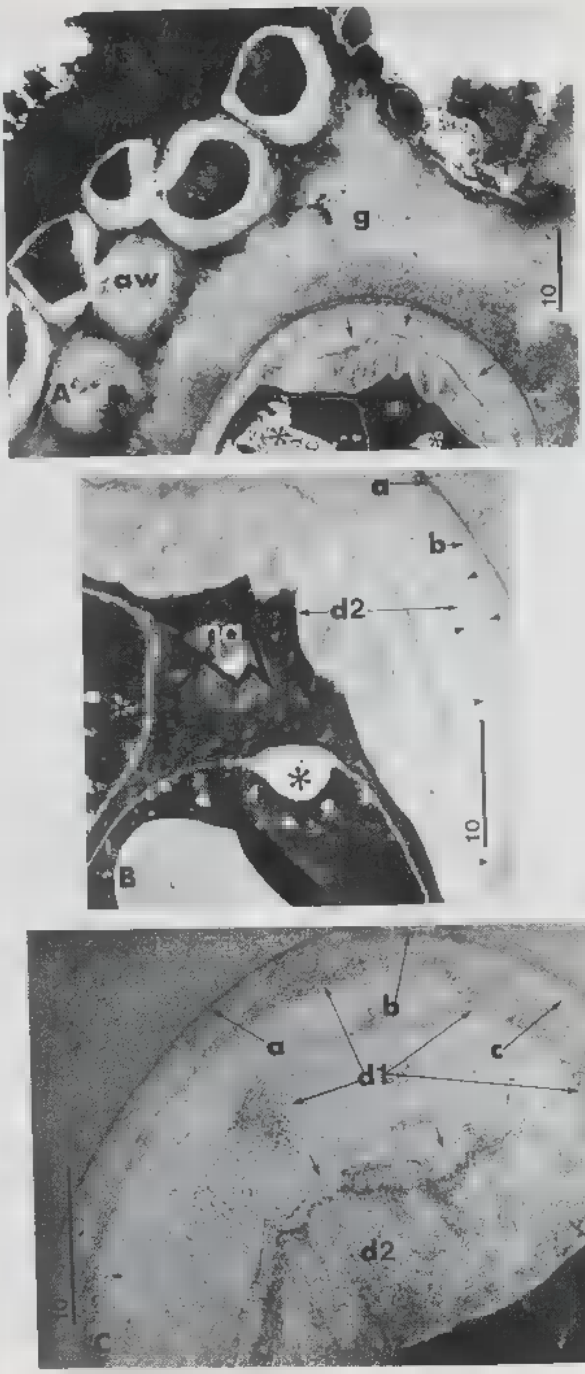
The use of the term « couche » to interpret the ascus wall introduced by BELLEMÈRE (1971) can be used to label the layers of the mature ascus wall. A comparison of the ascus in mid- or near midsection with a tangential section is made in order to give additional characterization to each Couche.

The encompassing primary wall is comprised of two layers seen and can be identified in the mature ascus as comprising Couches A and B (Fig. 6). Couche A is a densely staining external layer ; Couche B consists of a wall matrix with the same, but less dense, parallel-to-the-surface orientation of the wall components.

REACTIVE ASCUS WALL COMPONENT	IODINE TREATMENT			
	No Pretreatment		Pretreatment	
	IKI	Meltzer's	IKI	Meltzer's
Primary ascus wall	BB	b	---	---
Secondary ascus wall				
Dome	---	---	1b	1b
Inner surface	---	---	b	b

Table 1 — Amyloidity in the ascus of *Asterina carbonacea* Cooke. Protocol followed was that of Baral (1987). The color resulting from the application of iodine in IKI and Meltzer's solutions and with and without pretreatment of the ascus with KOH are tabulated (BB = blue in high and low iodine concentration ; · · · = no reaction ; b = blue ; 1b = light blue).

Tableau 1 – Réaction amyloïde de l'asque d'*Asterina carbonacea* Cooke. Tableau des colorations obtenues par les solutions d'iode dans l'IKI et de Meltzer, avec ou sans prétraitement de l'asque par KOH (protocole selon Baral, 1987 ; BB = bleu pour des concentrations en iode élevées et faibles ; · · · = aucune réaction ; b = bleu ; 1b = bleu clair).



The formation of the two-layered secondary ascus wall begins with the deposition of Couches C and D. Couche C is apparent as a zone between Couche B and Couche D, recognizable by an abrupt difference in the microfibrillar distribution pattern.

There are two regions of Couche D which are visible with the resolution of the electron microscope (Figs. 6-7) but not with the light microscope (Fig. 5). The Couche D layers are fully deposited prior to ascosporogenesis. Couche D1 is peripherally deposited on the inner surface of Couche C.

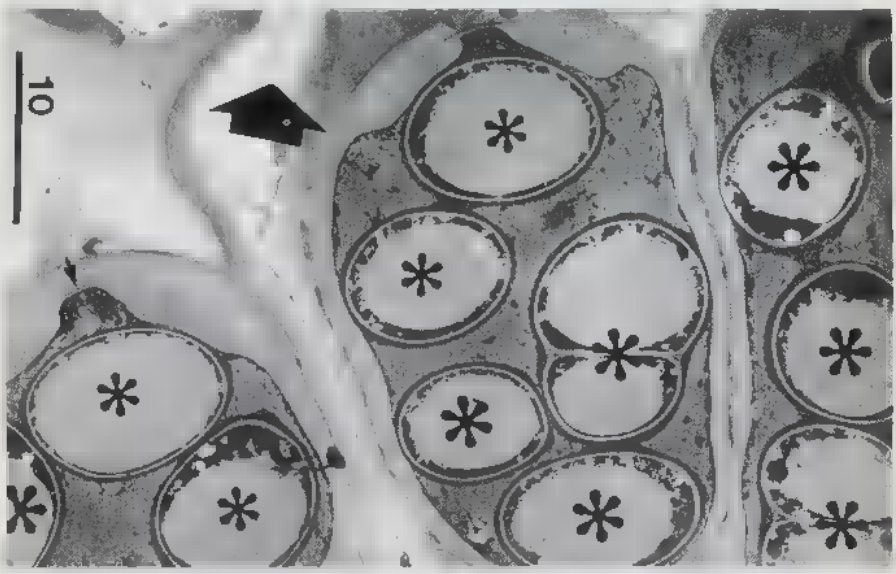


Fig. 7 — *Asterina carbonacea* Cooke. Asci (T.E.M.). Three mature asci with one or two cells of the bicellular ascospores (*) and the apical chamber (small arrows). A discharged ascus (large arrow pointing in the direction of the ascospore expulsion) can be identified in this hymenial section.

Fig. 7 — Asques d'*Asterina carbonacea* Cooke (M.E.T.). Trois asques mûrs contenant les ascospores bicellulaires (*) et la chambre apicale (petites flèches). Un asque vidé (large flèche pointée dans la direction de l'expulsion des ascospores) est visible sur cette coupe de l'hyménium.

Fig. 6 — *Asterina carbonacea* Cooke. Longitudinal sections in slightly off-center views (T.E.M.) A. View of tip of ascocarp near wall (aw), showing the tip of an ascus with ■ mature wall which contains two immature ascospores (*). Note the gelatineous matrix (g) surrounding the ascus. The arrows indicate the stress zone just above the tangential view of the nasse apicale which is formed during ascosporogenesis ■ the ascus accommodates the developing ascospores. B. An enlarged section of A. Couche A (a) and Couche B (b) are the outermost layers, with Couche C not well demonstrated. Couche D1 (d1) is indicated in the area outlined by the small stemless arrows with Couche D2 (d2) evident by the banded layers. The immature ascospores (*) lack walls. C. Oblique tangential section through ascus apex. Couches A-D (a, b, c, d) are demonstrable. Stressed layer created by wall reorientation during ascospore formation is seen in ■ lateral region above Couche D2 (small arrows).

Fig. 6 — Coupes longitudinales d'asques d'*Asterina carbonacea* Cooke (M.E.T.). A. Sommet de l'ascocarpe près de la paroi (aw), montrant l'apex de l'asque avec une paroi mûre qui entoure 2 ascospores immatures (*). Noter la matrice gélatineuse (g) entourant l'asque. Les flèches indiquent la zone de scission juste au-dessus de la nasse apicale (vue tangentielle) qui est formée pendant l'ascosporogenèse. B. Détail de A. La couche A (a) et la couche B (b) sont les couches extrêmes, la couche C est peu distincte. La couche D1 (d1) est la zone définie par les têtes de flèches et la couche D2 (d2) montre différentes strates. Les ascospores immatures (*) n'ont pas de paroi. C. Coupe tangentielle oblique dans l'apex de l'asque où l'on peut voir les couches A à D (a, b c, d). La zone de scission créée par la réorientation de la paroi au cours de la formation des ascospores est située dans la région latérale au-dessus de la couche D2 (petites flèches).

Couche D1 has the banded pattern of the bitunicate endotunica (REYNOLDS, 1971). Dark-staining microfibrils are embedded in the amorphous wall matrix with an orientation that is generally horizontal to the protoplast surface. Additionally, the embedded microfibrils form a reoccurring pattern of verticle bands. The peaks and valleys of undulations in the wall give definition to the band boundaries. Couche D1 forms a slight bulge (Fig. 6) at the lower edge of the Couche D2 dome and becomes thinner toward the ascus apex.

Couche D2 is deposited last and is found in the upper third of the ascus as a dome-shaped layer (Fig. 6). The microfibrillar deposition pattern of Couche D2 in asci with developing or mature ascospores has lines that run across and perpendicular to the vertical band zones (Figs. 6 A-B, 7). These lines have a discernibly different arching angle in the preascosporogenic and the post ascosporogenic ascus. There is ■ noticeable density in these lines immediately over the nasse apicale. These lines appear to be « stretch marks » formed during ascosporogenesis as a result of the stress in the wall layers induced during the accommodation of the developing ascospores.

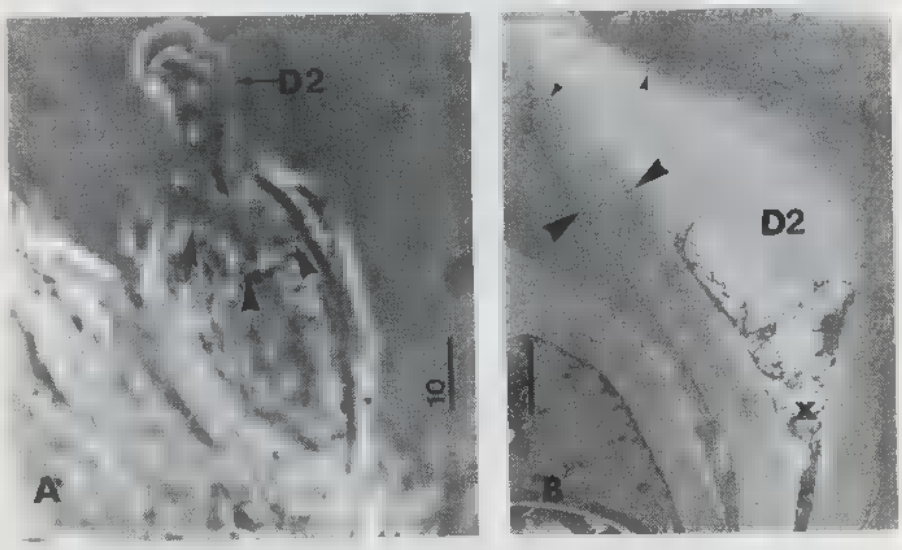


Fig. 8 — *Asterina carbonacea* Cooke. Comparative light and electron microscopic views of discharged asci. A. Ascus with wall extended beyond disrupted wall (arrows) (light microscope). This view demonstrates the possibility that the ascus can be interpreted as bitunicate at this resolution. As discerned from Fig. B, Couche D2 (D2) extends beyond the remaining portion of the wall (arrows). B. View similar to A of the ascus discharge apparatus with the resolution of the transmission electron microscope. The ascus breaks (small arrows) to allow an extension of the ascus wall which is formed from Couche D2. Couche D2 remains unseparated from the Couches A-D1 portion of the ascus wall (indicated between large arrows).

Fig. 8 — *Asterina carbonacea* Cooke. Comparaison des clichés de microscopie optique et électronique d'asques vidés. A. Asque montrant une paroi étirée au-dessus de la paroi déchirée (flèches) (microscopie optique). Ce cliché montre que l'asque peut être interprété comme bituniqué à ce grandissement. Comme on peut le voir sur la Fig. B, la Couche D2 (D2) s'étend au-delà des restes de la paroi (flèches). B. Vue correspondant à la Fig. A, avec la résolution du microscope électronique à transmission. L'asque s'ouvre (petites flèches) pour permettre une extension de la paroi ascale, formée à partir de la couche D2. La Couche D2 reste liée aux Couches A-D1 de la paroi ascale (portion indiquée entre les grandes flèches).

An apical extension of the protoplast is maintained from the beginning of the Couche D2 wall deposition (Figs. 5-9). The usual position of the apical extension is in ■ line on the approximate axis of the ascus. The structural integrity of the ascus apex extension and the Couche D2 socket into which it fits can be demonstrated in squash mounts in which the protoplast is mechanically separated from the ascus cell wall. Occasionnally a slight separation occurs immediately above the ascus apex extension, forming a spacial artifact.

The inner-wall surface interface with the apical cytoplasmic extension is undulate as a result of the underlying surface configuration of the banded pattern Couche D1. This feature can be identified with a through-focus examination which demonstrates an overlapping array of curving focus-planes formed by the band boundaries. The spiral rods formed from the optical properties of the protoplast inner-wall surface can be seen in some microscope preparations.

Ascosporogenesis occurs after the formation of the ascus wall is completed. Most of the asci are eight-spored, although some were seen to have only four pigmented ascospores. The ascus shape changes as the ascospore initials are formed, presumably during meiosis and especially during the latter stages when the ascospore wall is deposited.

With the formation of the ascospores, the ascus assumes ■ more rounded shape. During this stage, the Couche D layers undergo further modification as the ascus stretches to accommodate the developing ascospores (Figs. 5 B-C, 6). At ascospore release, the outer wall layer splits (Figs. 8, 9). The Couche D2 layer extends as a tube.

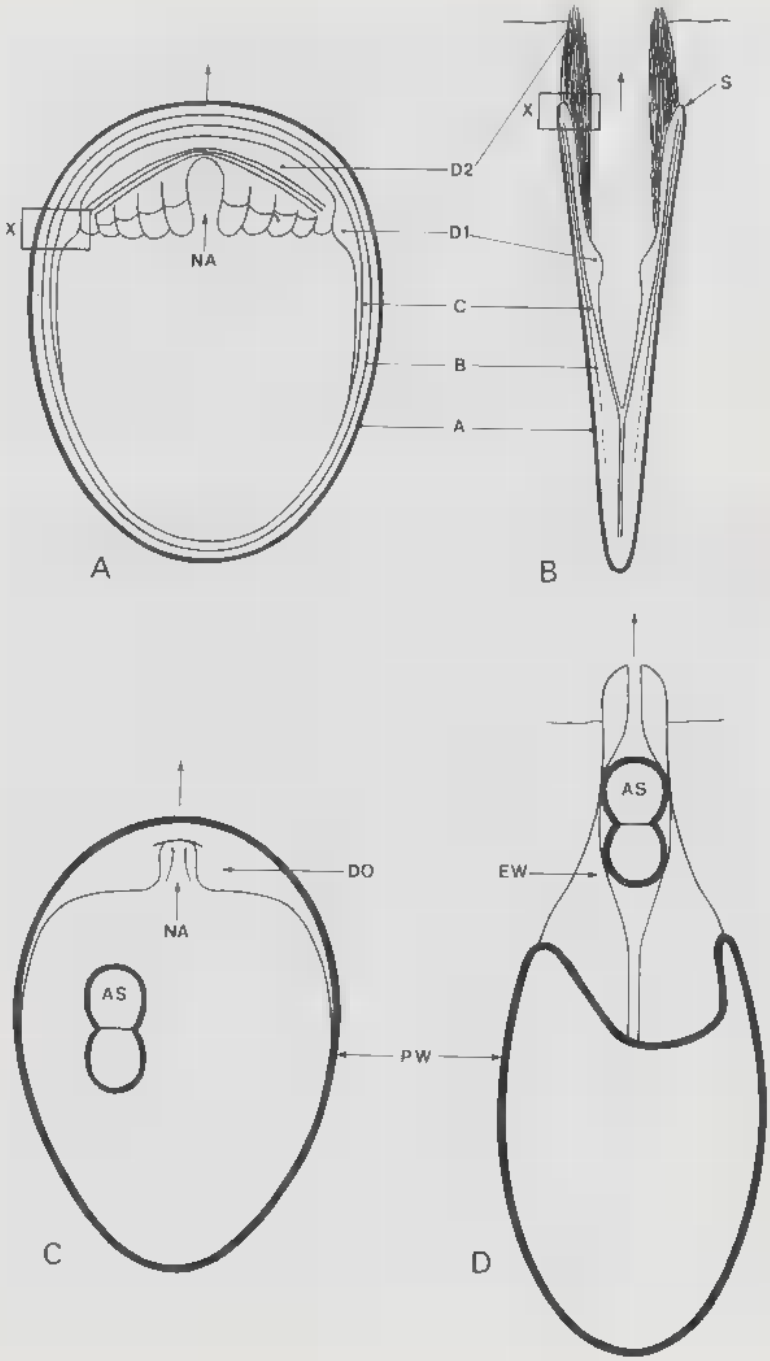
Contrary to the impression gained from light microscopic viewing (Fig. 8 A), there is no separation of the Couche D2 layer from the other wall layers (Figs. 8 B, 9). The orientation of the microfibrils in the wall matrix after ascospore ejaculation indicate no upward displacement from the original interface of Couche D1 and Couche D2 (Fig. 8 B).

The ascospores leave the ascus in single-chain formation (Fig. 9 D) and exit the extended wall via the unmodified, open apical end of the surrounding tube (Figs. 8-9). The expended asci, consisting of the collapsed lower wall and the tubular extension of the secondary wall, often remain in situ in the hymenium.

DISCUSSION

The results of this study support a recognition of the asterinaceae and related families as a sister group to the loculoascomycetes which were defined by LUTTRELL (1955) as « Ascis bitunicatis, in ascostromate evolutis ». The *Asterina* ascus cannot be equated with the bitunicate ascus as defined by LUTTRELL (1951) and implicated by ERIKSSON (1984). The ascus structure is similar to that described by HONEGGER (1978) as the « type eu-archaeascé of LETROUIT-GALINOU, 1973 » that ERIKSSON (1981) termed « rostrate ».

The asci from *Asterina correicola* Cooke & Massee and *Asterina* sp. were said to develop from proliferating croziers (SWART, 1969). Their development and the sympodially produced asci of *A. carbonacea* originate in a manner suggestive of the mode of ascus formation discussed by PARGUEY-LEDUC (1977) as « Dangeardies simples ». The asci of *Placoasterella baileyi* were found to arise from a terminal cell of the fertile member of ■ duel hyphal system making up the asco-



carp (TYSON & GRIFFITHS, 1976a). The ascus-bearing cell was noted to ultimately be connected to a binucleate penultimate cell.

THEISSEN (1913) described the ascus of the genus *Asterina* species as having ■ double tunica with the inner sporesack tightly surrounding the spores and the outer, mostly very thin, slimy tunica. The illustrations of the ascus in this early review of the genus *Asterina* indicate a thick nonlayered wall delimiting the ascus ; no details of the apical region were provided. The outer tunica was likely a reference to the reaction that was obtained when iodine was applied to the ascus in several species.

Sudan Black B stains for total lipids (O'BRIEN & McCULLY, 1981). The blue reaction in asci without KOH pretreatment possibly indicates the presence of isolichenin (CULBERSON, 1969). The light blue staining of the ascus dome and the darker blue reaction of the inner surface of the secondary wall with KOH pretreatment might be indicative of curled molecular segments presents in the secondary wall material which is destined to undergo stretching (BARAL, 1987).

Spore ejaculation triggers the phenomenon which is the historic basis of characterization of the bitunicate ascus (LUTTRELL, 1951). The primary wall ruptures in the apical region and slides down the secondary wall which stretches into a tube through which the ascospores are distributed (REYNOLDS, 1971 ; BECKETT & al., 1974 ; BEZERRA & KIMBROUGH, 1982 ; PARGUEY-LEDUC, 1977 ; PARGUEY-LEDUC & JANEX-FAVRE, 1982 ; BELLEMÈRE & HAFELLNER, 1982 ; NIYO & al., 1986).

Fig. 9 — Diagramatic representations of the *Asterina carbonacea* Cooke wall structure as seen in light and electron microscopic views. A comparison of the undischarged ascus with the ascus with the inner wall extended. A. The undischarged ascus in an electron microscopic view. B. The discharged ascus in an electron microscopic view. At ascospore discharge, the ascus wall will rupture (X) near where Couche D1 forms an enlargement and adjacent to the Couche D2 or « apical dome » portion of the ascus. Stress lines are formed over the nasse apicale (NA) in Couche D2. Couche D2 extends beyond the remainder of the ascus wall (S) at the rupture (X). C. The undischarged ascus in ■ light microscopic view. The primary wall (PW) encloses the secondary wall which is largely deposited ■ an apical dome (DO) where an apical chamber with a nasse apicale (NA) occurs. The bicelled ascospores (AS) are contained in an oval chamber shaped during ascosporogenesis by the stretching of the secondary wall material. D. The discharged ascus in a light microscopic view. The primary wall (PW) splits to allow the extension of the inner elastic wall (EW). The ascospores (AS) distend the inner canal of the extended wall as they are discharged from the lower portion of the ascus. The arrows indicate direction of ascospore discharge in the undischarged ascus. A = Couche A, B = Couche B, C = Couche C, D1 = Couche D1, D2 = Couche D2.

Fig. 9 — Représentations schématiques de la structure de la paroi d'*Asterina carbonacea* Cooke, observée au microscope optique et électronique. Comparaison d'un asque fermé et d'un asque dont la paroi interne est étirée. A. Asque fermé, en microscopie électronique. B. Asque ouvert, en microscopie électronique. A l'éjection de l'ascospore, la paroi de l'asque se rompt (X) à l'endroit où la Couche D1 est élargie et adjacente à la couche D2 (« dôme apical » de l'asque). Des lignes de scission se créent dans la couche D2, au-dessus de la nasse apicale (NA). La Couche D2 s'étire au-delà de la paroi ascale (S) au point de rupture (X). C. Asque fermé, en microscopie optique. La paroi primaire (PW) entoure la paroi secondaire qui forme un dôme apical (DO) où apparaît une chambre apicale avec une nasse apicale (NA). Les ascospores bicellulaires (AS) sont contenues dans une chambre ovale formée au cours de l'ascosporogenèse par une extention de la paroi secondaire. D. Asque ouvert, en microscopie optique. La paroi primaire (PW) se divise pour permettre l'extension de la paroi élastique intérieure (EW). Les ascospores (AS) distendent le canal interne de la paroi lorsqu'elles sont expulsées de la partie inférieure de l'asque. Les flèches indiquent la direction de l'éjection des ascospores dans l'asque fermé. A = Couche A, B = Couche B, C = Couche C, D1 = Couche D1, D2 = Couche D2.

The technique stressed by BELLEMÈRE (1971) and other French workers for observations on the ascus wall structure at the resolution of the electron microscope is that attributed to THIÉRY (1967) ; the deposition patterns of the polysaccharides comprising the ascus wall were enhanced with thiocarbohydrazide or thiosemicarbazide and with silver proteinate staining. On the other hand, other recent workers such as HONEGGER (1985) and NIYO & al. (1986) were able to discern the wall patterns with the use of uranyl acetate and lead citrate, as was done in this study. I believe that the Thiéry Technique is very useful for improving the constrast of micrographs but is not a neccessity for the interpretation of the ascus wall structure.

The *Asterina* ascus appears bitunicate at the resolution of the light microscope (Fig. 9 A). The formation of this ascus follows the same sequential pattern as that of the bitunicate ascus (REYNOLDS, 1971). Yet, the wall structure is not the same (Fig. 9). The distribution of the banded pattern secondary wall, Couche D (BELLEMÈRE, 1971), is different than that of the bitunicate ascus with separable wall. The Couche D layer is characterized by the vertical band pattern of the bitunicate ascus, yet this secondary wall material is measurably more concentrated in the apical portion of the ascus. The bulge of Couche D1 at the point where Couche D2 begins is suggestive of the thickening of the wall found by SAMUELSON & KIMBROUGH (1978) in *Thelebolus polysporus* (Marst.) Otani & Kanzawa. Unlike the ascus of that species, the upper portion of the *Asterina* ascus wall does not undergo a separation into an eclotunica and an endotunica during spore ejaculation. Couche D2 stretches into a tube without separation from the rest of the wall. The ascospores are dispersed through the resultant channel in the extension of the wall.

The presence of density lines that run parallel to the periphery of the ascus and across the vertical columns of the banded pattern are an indicator of the reorientation of the secondary wall material during ascospore formation. The increase in density of these lines just above the nasse apicale might result from the focus of stress in the stretching ascus at that pivotal point ; the meniscus reported by ERIKSSON (1981) likely results from mechanical disruption of the wall at this stress point. Applying the observation of MARTIN & al. (1976) that the « contorted plasmalemma » is related to an increase in the ascus volume, I suggest that Couche D1 absorbs the changes in the ascus wall during ascosporogenesis while Couche D2 functions during ascospore dispersal.

The definition of an ascostroma in HAWKSWORTH & al. (1983) is a stroma in or on which asci are produced ; the term was said by them to be usually restricted to groups with ascolocular ontogeny. These editors give the definition of Ascoloculares as ascomycetes having asci developing in cavities in a preformed stroma. An alternate term, pseudothecium, was defined as an ascostromatic ascocarp having asci in numerous unwalled locules. The ascocarp formation by *Asterina*, including *A. carbonacea*, was investigated by GAILLARD (1893) who found that the fruitbody developed from a hyphal cell before the appearance of the ascus. RYAN (1926) confirmed this sequence in eighteen illustrated species of *Asterina*.

The ascoma of *Asterina* begins from a hyphal cell that, in turn, is the source of a duel hyphal system. The first developed system forms the ascocarp, and the second forms the ascogenous system. Therefore the ascoma is an ascostroma sensu LUTTRELL (1951).

The systematic implication of this study is that broached by LUTTRELL (1955) in his discussion of the exceptions (the Coryneliales and the Coronophorales) to the definition of the subclass Loculoascomycetes because of the occurrence of a nonbitunicate ascus in an ascostroma. Extrapolating the results of this study, the groups pointed out by LUTTRELL (1973) as having an ascus that is « globose to broad oblong or clavate (less than 3 times as long as broad) » apparently also comprise a major exception to his definition. This ascus type is found in the Asterinaceae as well as the Seuratiaceae and possibly other ascostromatic families such as the Philipsclliaceae. These families are predicted as nonbitunicate-ascus ascostromatic, but not Euascomycetes, sister taxa that are phylogenetically near the ascostromatic ascomycetes with a bitunicate ascus.

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